

Note to readers with disabilities: *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to [508 standards](#) due to the complexity of the information being presented. If you need assistance accessing journal content, please contact ehp508@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.

Supplemental Material

Xenobiotic Exposure and Migraine-Associated Signaling: A Multimethod Experimental Study Exploring Cellular Assays in Combination with *Ex Vivo* and *In Vivo* Mouse Models

Rikke H. Rasmussen, Sarah L. Christensen, Kirstine Calloe, Brian Skriver Nielsen, Anders Rehfeld, Thomas E. Taylor-Clark, Kristian A. Haanes, Olivier Taboureau, Karine Audouze, Dan A. Klaerke, Jes Olesen, and David M. Kristensen

Table of Contents

Figure S1. Cytotoxicity of hTRPA1-HEK cells after pentachlorophenol exposure. One day prior to measuring, cells were seeded at a density of 25,000 cells/well (90 µL). Cells were treated with pentachlorophenol (PCP) in increasing concentrations (1 µM, 10 µM and 100 µM) for 1 h. The viability reagent alamar blue (10 µL) was added to the cells and incubated for 1 h. Data is normalized to the vehicle (0.1% DMSO). Control is 10 µM ionomycin. Data is presented as means ± SD. One-way ANOVA with Dunnett's correction for multiple comparisons using vehicle as the reference. Numeric data is found in Table S4.

Figure S2. Molecular modeling of acrolein docking in hTRPV1 and hTRPA1. Docking of acrolein into the binding pocket of hTRPV1 and hTRPA1 using the same protocol and parameters as for PCP. **(A)** 2D representation of the docking pose of acrolein in hTRPV1. **(B)** 2D representation of the docking pose of acrolein in hTRPA1. **(A-B)** The pink spheres are polar residues (\$). The green spheres are hydrophobic residues (#). Red borders are for acidic (*) and blue for basic (†). The blue shadows are ligand exposure and receptor exposure.

Table S1. Summary data for Figure 1A.

Table S2. Summary data for Figure 1B.

Table S3. Summary data for Figure 1C.

Table S4. Summary data for Figure S1.

Table S5. Summary data for Figure 2C & 2F.

Table S6. Summary data for Figure 2I.

Table S7. Summary data for Figure 4A. Measured data can be found in Table S11.

Table S8. Summary data for Figure 4B. Measured data can be found in Table S12.

Table S9. Summary data for Figure 4C. Measured data can be found in Table S13.

Table S10. Summary data for Figure 4D. Measured data can be found in Table S14.

Table S11. Non-normalized data for Figure 4A.

Table S12. Non-normalized data for Figure 4B.

Table S13. Non-normalized data for Figure 4C.

Table S14. Non-normalized data for Figure 4D.

Table S15. Summary data for Figure 4E.

Table S16. Summary data for Figure 5A.

Table S17. Summary data for Figure 5B.

Table S18. Summary data for Figure 5C.

Table S19. Summary data for Figure 5D.

Table S20. Summary data for Figure 5E.

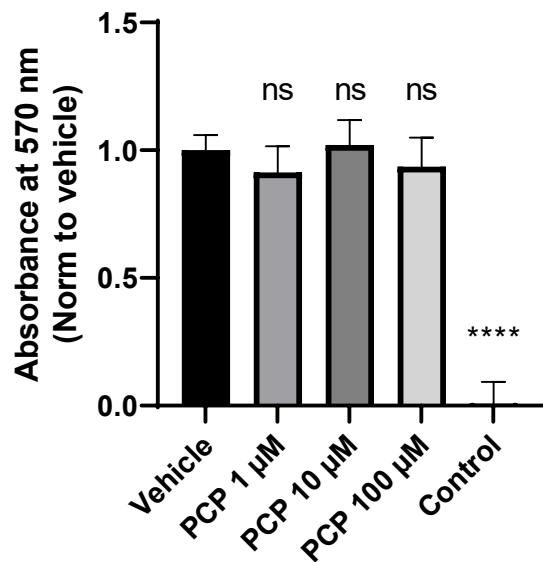


Fig. S1: Cytotoxicity of hTRPA1-HEK cells after pentachlorophenol exposure. One day prior to measuring, cells were seeded at a density of 25,000 cells/well (90 μ L). Cells were treated with pentachlorophenol (PCP) in increasing concentrations (1 μ M, 10 μ M and 100 μ M) for 1 h. The viability reagent alamar blue (10 μ L) was added to the cells and incubated for 1 h. Data is normalized to the vehicle (0.1% DMSO). Control is 10 μ M ionomycin. Data is presented as means \pm SD. One-way ANOVA with Dunnett's correction for multiple comparisons using vehicle as the reference. Numeric data is found in Table S4.

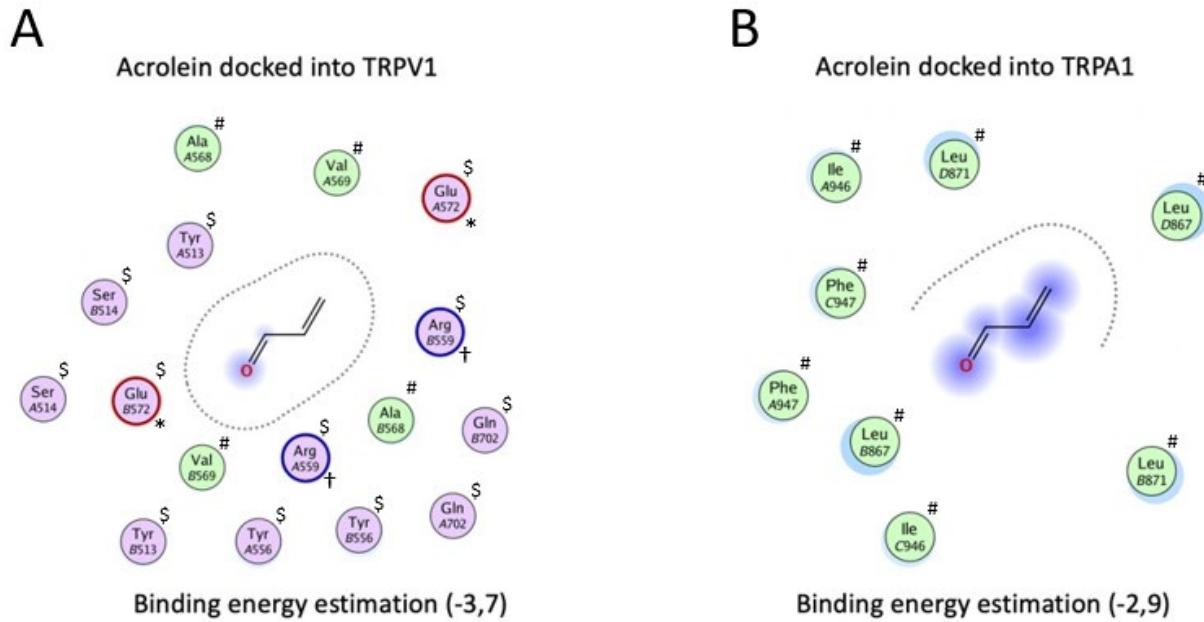


Fig. S2: Molecular modeling of acrolein docking in hTRPV1 and hTRPA1. Docking of acrolein into the binding pocket of hTRPV1 and hTRPA1 using the same protocol and parameters as for PCP. (A) 2D representation of the docking pose of acrolein in hTRPV1. (B) 2D representation of the docking pose of acrolein in hTRPA1. (A-B) The pink spheres are polar residues (\$). The green spheres are hydrophobic residues (#). Red borders are for acidic (*) and blue for basic (†). The blue shadows are ligand exposure and receptor exposure.

Table S1 Summary data for Figure 1A.

Group	Chemical	Abbreviation	Control cells		hTRPA1-HEK		hTRPV1-HEK	
			Mean (%)	SD	Mean (%)	SD	Mean (%)	SD
Phthalates	Dimethyl phthalate	DMP	0.88	0.74	-2.00	1.96	-0.58	1.03
	Diethyl phthalate	DEP	0.32	0.45	19.18	9.79	-0.98	0.94
	Di-n-pentyl phthalate	DPP	1.01	0.72	0.33	1.67	-0.18	0.94
	Di-n-butyl phthalate	DBP	0.20	0.48	1.31	1.57	0.87	1.73
	Di-iso-butyl phthalate	DiBP	0.38	0.62	4.21	1.41	-0.60	0.99
	Benzyl butyl phthalate	BBP	0.38	0.66	-0.76	1.76	-0.29	0.78
	Di-n-nonyl phthalate	DNP	0.41	0.39	-0.87	1.98	-0.08	0.86
	Di-iso-nonyl phthalate	DiNP	-0.06	0.33	-0.96	1.51	-0.58	0.95
	Di-2-ethylhexyl phthalate	DEHP	-0.40	0.25	0.70	1.14	-0.56	1.05
	Di-n-octyl phthalate	DOP	0.12	0.96	1.66	2.10	0.45	0.43
Parabens	4-hydroxybenzoic acid	HBA	-0.78	0.33	-1.05	1.53	-0.85	0.95
	Methyl paraben	MPa	-0.94	0.40	-1.28	1.40	-1.09	1.03
	Ethyl paraben	EPa	0.44	0.42	-0.45	1.00	-0.22	0.66
	Propyl paraben	PPa	-0.04	0.44	18.53	11.90	-0.67	0.57
	Butyl paraben	BPa	1.07	0.66	22.94	9.13	-0.25	0.55
	Iso-butyl paraben	iBPa	-0.12	0.55	22.30	7.02	-0.48	0.75
	Benzyl paraben	BzPa	0.94	0.26	34.19	8.58	-0.02	1.19
UV filters	Benzophenone 3	BP3	-1.21	0.63	13.37	6.12	-0.96	0.56
	Benzophenone 4	BP4	0.05	0.30	-1.26	1.31	-0.91	1.07
	Benzophenone 7	BP7	-1.18	0.27	16.42	8.28	-1.39	2.35
	Benzophenone 12	BP12	-0.15	0.31	0.55	1.54	-0.58	0.54
	Octinoxate	OMC	-0.54	0.33	0.79	1.68	-0.36	1.03
	4-methylbenzylidene camphor	4-MBC	-0.29	0.85	-0.67	1.13	-0.33	0.73
Naturally occurring hormones or hormone-like compounds	Diethylstilbestrol	DES	3.15	1.53	3.67	2.12	2.85	2.38
	Nonyl phenol	NNP	0.66	0.68	29.88	6.59	0.18	0.99
	Bisphenol A	BPA	0.05	0.15	11.00	7.96	0.28	0.56
	Dihydrotestosterone	DHT	-0.93	0.31	-1.32	1.73	-0.12	0.27
	Testosterone	Testo	-0.19	0.29	-0.94	1.53	0.13	0.24
Mild analgesics and precursors	Paracetamol	Para	-0.09	0.34	0.40	0.54	0.16	0.39
	Aspirin	ASA	0.11	0.88	0.00	0.58	0.26	0.17
	Ibuprofen	Ibu	-0.30	0.76	-0.42	1.44	-0.09	0.40
	Aniline	Ani	-0.15	0.65	-1.08	1.13	0.11	0.30
Polyfluorinated compounds	Perfluorooctanesulfonic acid	PFOS	2.15	1.48	2.10	1.73	-0.03	0.81
	Perfluorooctanoic acid	PFOA	0.53	0.33	-0.02	2.61	0.00	0.28
Controls	Supercinnamaldehyde	SCA	-0.16	0.73	72.69	6.47	-0.34	0.59
	Capsaicin	Caps	-0.84	0.69	-2.07	0.72	30.4	3.02

Table S2 Summary data for Figure 1B

Group	Chemical	Abbreviation	Control cells		hTRPA1-HEK		hTRPV1-HEK	
			Mean (%)	SD	Mean (%)	SD	Mean (%)	SD
Pesticides or metabolites	Chlorpyrifos	-	-0.02	0.19	4.52	3.43	-0.78	0.54
	Prosulfocarb	-	0.00	0.52	11.72	6.07	-1.59	0.92
	Fipronil Sulfone	-	1.25	0.86	5.23	8.01	-0.79	1.42
	Trifluralin	-	-0.11	0.30	-2.26	1.10	-1.11	1.34
	Endosulfan	-	-0.01	0.43	15.39	5.16	-1.32	1.19
	Hexachlorophene	HCP	2.92	0.53	46.96	4.88	0.74	1.50
	Imazalil	-	-0.33	0.19	-2.30	0.91	-1.26	0.99
	Oxadiazon	-	-0.57	0.25	-2.06	1.01	-1.50	0.98
	Lindane	-	-0.42	0.92	28.53	6.42	-0.80	1.38
	Pentachlorophenol	PCP	0.88	0.81	34.36	4.14	-0.20	0.74
	Prochloraz	-	-0.81	0.54	-1.82	1.02	-0.93	1.19
	Cypermethrin	-	0.68	0.40	-2.33	0.64	-0.86	1.21
	Permethrin	-	0.32	0.69	-1.99	0.71	-0.72	1.31
	Deltamethrin	-	0.12	0.70	-0.33	1.74	-0.76	1.32
Controls	Supercinnamaldehyde	SCA	-0.16	0.73	72.69	6.47	-0.34	0.59
	Capsaicin	Caps	-0.84	0.69	-2.07	0.72	30.41	3.02

Table S3 Summary data for Figure 1C

Group	Chemical	Abbreviation	hTRPA1-HEK	
			Mean (%)	SD
Pesticides	Pentachlorophenol	PCP	42.85	2.71
	Hexachlorophene	HCP	58.83	5.34

Table S4 Summary data for Figure S1

Treatment	Mean	SD	P values (compared to vehicle)
Vehicle	1.00	0.06	-
PCP 1 µM	0.91	0.10	0.33
PCP 10 µM	1.02	0.098	0.99
PCP 100 µM	0.94	0.11	0.59
Control	0.0082	0.085	<0.0001

Table S5 Summary data for Figure 2C & 2F

Control		PCP peak		PCP after 50 s		PCP + HC		P values			
Mean (nA)	SEM	Mean (nA)	SEM	Mean (nA)	SEM	Mean (nA)	SEM	Control vs. PCP peak	PCP peak vs. PCP after 50 s	PCP peak vs. PCP + HC	
hTRPA1- HEK	0.5236	0.2586	6.3713	1.279	0.647	0.4256	0.0838	0.0403	0.0031	0.0048	0.0022
Control cells	0.3901	0.1689	0.3274	0.1276	-	-	0.3798	0.1304	0.66	-	0.89

Table S6 Summary data for Figure 2I

Control		PCP 10 µM		P values	
Mean (nA)	SEM	Mean (nA)	SEM	Control vs. PCP 10 µM	
TG neurons	-40.30	9.54	-463.42	226.9	0.0020

Table S7 Summary data for Figure 4A. Measured data can be found in Table S11.

SCA		Vehicle		P values	
PCP concentration	Mean	SEM	Mean	SEM	
Basal	1.00	0.00	1.00	0.00	-
1 µM	1.53	0.36	1.25	0.31	0.56
10 µM	3.26	0.80	1.25	0.30	0.09
100 µM	8.89	1.88	1.07	0.25	0.033

Table S8 Summary data for Figure 4B. Measured data can be found in Table S12.

SCA		Vehicle		P values	
PCP concentration	Mean	SEM	Mean	SEM	
Basal	1.00	0.00	1.00	0.00	-
1 µM	0.87	0.08	1.16	0.10	0.119
10 µM	1.00	0.07	1.05	0.16	0.780
100 µM	1.54	0.21	1.04	0.11	0.134

Table S9 Summary data for Figure 4C. Measured data can be found in Table S13.

PCP		Vehicle		P values	
PCP concentration	Mean	SEM	Mean	SEM	
Basal	1.00	0.00	1.00	0.00	-
1 µM	1.11	0.07	1.08	0.09	0.814
10 µM	1.51	0.11	0.83	0.06	0.0005
100 µM	4.95	1.24	0.92	0.06	0.028

Table S10 Summary data for Figure 4D. Measured data can be found in Table S14.

PCP concentration	PCP		Vehicle		P values
	Mean	SEM	Mean	SEM	
Basal	1.00	0.00	1.00	0.00	-
1 µM	1.12	0.09	1.05	0.06	0.788
10 µM	1.14	0.12	1.25	0.14	0.788
100 µM	1.51	0.25	1.09	0.18	0.495

Table S11 Non-normalized data for Figure 4A

SCA concentration	SCA (CGRP pg/mL)							Vehicle (CGRP pg/mL)					
	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	Mouse 7	Mouse 8	Mouse 9	Mouse 10	Mouse 11	Mouse 12	Mouse 13
Basal	38.9	162.1	85.1	45.4	96.2	77.7	46.5	49.7	71.2	189.2	60.9	91.4	40.9
1 µM	66.0	126.0	124.9	157.7	58.4	96.1	66.9	126.8	109.3	58.6	76.5	64.3	46.0
10 µM	193.0	406.9	547.5	112.1	76.3	84.3	213.3	48.8	116.8	106.2	65.0	64.3	103.4
100 µM	615.9	1401.0	998.5	230.7	156.6	282.0	729.2	104.8	82.1	72.5	82.9	56.0	32.7

Table S12 Non-normalized data for Figure 4B

SCA concentration	SCA (CGRP pg/mL)						Vehicle (CGRP pg/mL)					
	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	Mouse 7	Mouse 8	Mouse 9	Mouse 10	Mouse 11	Mouse 12
Basal	101.6	59.1	84.4	73.0	49.5	43.2	71.3	106.7	87.6	54.3	36.2	58.7
1 µM	94.7	55.9	64.6	42.5	56.1	36.6	108.1	100.1	96.9	53.3	50.7	59.8
10 µM	99.6	59.1	60.6	83.7	61.2	38.7	50.5	85.1	74.1	83.2	56.7	48.8
100 µM	166.0	147.4	99.0	113.1	61.4	49.5	74.7	132.5	80.1	45.8	53.3	42.5

Table S13 Non-normalized data for Figure 4C

PCP concentration	PCP (CGRP pg/mL)							Vehicle (CGRP pg/mL)						
	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	Mouse 7	Mouse 8	Mouse 9	Mouse 10	Mouse 11	Mouse 12	Mouse 13	Mouse 14
Basal	97.0	110.4	104.9	61.4	61.3	71.0	47.4	45.7	83.4	50.3	55.3	56.9	46.1	49.9
1 µM	115.0	94.7	125.8	59.4	56.3	104.9	51.6	53.1	93.1	46.5	41.8	73.0	48.1	67.7
10 µM	180.5	146.6	188.7	81.2	65.5	123.6	60.1	78.5	61.7	35.6	41.5	57.7	35.3	50.1
100 µM	1071.0	211.7	1002.0	306.5	85.9	213.6	202.8	154.6	73.3	44.1	51.3	53.8	33.4	57.0

Table S14 Non-normalized data for Figure 4D

PCP concentration	PCP (CGRP pg/mL)							Vehicle (CGRP pg/mL)					
	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6	Mouse 7	Mouse 8	Mouse 9	Mouse 10	Mouse 11	Mouse 12	Mouse 13
Basal	96.9	86.1	120.0	68.5	91.8	48.8	45.5	125.1	42.9	76.9	61.3	48.9	35.3
1 μM	85.7	104.7	124.9	57.7	138.0	62.5	48.2	118.9	54.6	83.8	52.0	53.9	36.9
10 μM	102.3	120.2	81.5	97.8	76.1	73.0	48.7	129.7	83.0	74.6	80.2	56.7	39.1
100 μM	227.1	163.8	113.1	96.4	53.9	107.7	53.2	50.1	74.2	65.2	64.6	59.7	45.3

Table S15 Summary data for Figure 4E

PCP concentration	Trpa1 ^{-/-}		WT + CGRP ab		WT		P values	
	Mean (%)	SEM	Mean (%)	SEM	Mean (%)	SEM	Trpa1 ^{-/-} vs. WT	WT + CGRP ab vs. WT
10 ⁻⁷ M	96.97	5.54	98.60	4.81	94.05	7.11	0.99	0.99
3 × 10 ⁻⁷ M	100.68	8.66	98.99	10.93	90.45	7.88	0.25	0.79
10 ⁻⁶ M	98.99	4.51	82.94	9.87	84.62	6.17	0.05	0.99
3 × 10 ⁻⁶ M	92.05	1.74	60.36	3.27	38.27	5.24	<0.0001	0.033
10 ⁻⁵ M	67.99	4.45	20.22	3.83	9.14	1.07	<0.0001	0.55
3 × 10 ⁻⁵ M	8.07	0.39	-0.95	3.39	4.59	0.49	0.98	0.97

Table S16 Summary data for Figure 5A

	PCP		Vehicle		P values
	Mean	SEM	Mean	SEM	
Baseline	1.18	0.05	1.11	0.03	0.598
2 h	0.91	0.08	1.17	0.05	0.0430
4 h	0.86	0.09	1.15	0.07	0.0598

Table S17 Summary data for Figure 5B

	PCP		Vehicle		P values
	Mean	SEM	Mean	SEM	
Day 1	1.18	0.05	1.11	0.03	0.7818
Day 5	1.00	0.05	1.10	0.05	0.6182
Day 6	0.75	0.09	1.06	0.08	0.0542
Day 8	0.71	0.08	0.97	0.06	0.0445
Day 10	0.74	0.10	1.06	0.06	0.0494

Table S18 Summary data for Figure 5C

	PCP (Median)	Vehicle (Median)	Saline (Median)	Midazolam (Median)	P values
2 h	150 s	150 s	-	-	0.969
4 h	150 s	150 s	-	-	0.969
Controls	-	-	150 s	44.5 s	<0.0001

Table S19 Summary data for Figure 5D

	Vehicle - Trpa1^{-/-}		PCP - Trpa1^{-/-}		PCP - WT		P values	
	Mean	SEM	Mean	SEM	Mean	SEM	Vehicle Trpa1 ^{-/-} vs PCP Trpa1 ^{-/-}	Vehicle Trpa1 ^{-/-} vs PCP WT
Baseline	1.02	0.06	0.94	0.06	1.20	0.06	0.57	0.07
2 h	1.02	0.06	1.07	0.08	0.59	0.05	0.82	<0.0001
4 h	0.98	0.07	1.09	0.06	0.56	0.07	0.38	0.0004

Table S20 Summary data for Figure 5E

	Vehicle - Trpa1^{-/-}	PCP - Trpa1^{-/-}	PCP - WT	Saline	Midazolam	P values			
	Median	Median	Median	Median	Median	Vehicle Trpa1 ^{-/-} vs PCP Trpa1 ^{-/-}	Vehicle Trpa1 ^{-/-} vs PCP WT	PCP Trpa1 ^{-/-} vs PCP WT	Saline vs. Midazolam
2 h	150 s	150 s	150 s	-	-	0.5046	>0.9999	>0.9999	-
4 h	150 s	150 s	150 s	-	-	>0.9999	>0.9999	0.9363	-
Controls	-	-	-	150 s	38 s	-	-	-	<0.0001